# Endolymphatic Sac Carcinoma *In Situ* in a Tokay Gecko (*Gekko gecko*)

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**Abstract:** An 11-year-old female tokay gecko (*Gekko gecko*) was presented for bilateral asymmetric swellings of the cervical region. Baseline hematologic and plasma biochemical analyses demonstrated anemia, hypercalcemia, hyperphosphatemia, and hypoalbuminemia. Whole-body radiographs revealed a mass effect and irregular mineralization in the region of the left endolymphatic sac. Fine needle aspiration of this region revealed many elongated calcium-based crystals with pointed ends, histiocytic inflammation, and clusters of epithelial cells, consistent with hyperplasia or neoplasia of the epithelium of the endolymphatic sac. The crystals were shown to be calcium carbonate by infrared crystallography. Given the advanced state of disease and inability to completely excise the mass due to the intimate association between the meninges and endolymphatic sac, the gecko was euthanized. Gross necropsy and histopathologic evaluation revealed carcinoma *in situ* of the left endolymphatic sac. To our knowledge, this is the first report of neoplasia of the endolymphatic sac in a reptile.

**Key Words:** carcinoma, chalk gland, endolymphatic sac, *Gekko gecko*, neoplasia, tokay.

## Introduction

The tokay gecko (*Gekko gecko*) is a member of the family Gekkonidae and is one of the largest extant members of the genus *Gekko* (Manthey and Grossmann, 1997; Junior *et al.*, 2015). This arboreal species is nocturnal, carnivorous and insectivorous, and displays sexual dimorphism, with males being larger than females (Aowphol *et al.*, 2006). The tokay gecko has been widely used in research, is popular in the pet trade and pest control, and has medicinal and ritualistic purposes (Junior *et al.*, 2015). Although the natural distribution of the tokay gecko is throughout Southeast Asia, this species is also considered to be invasive in many regions of the world, including the Caribbean, the United States, Belize, Madagascar, and Brazil (Lever, 2003; Aowphol *et al.*, 2006; Junior *et al.*, 2015). The tokay gecko has not been assessed by the International Union for Conservation of Nature (IUCN) Red List of Endangered Species; although it is considered common, the status of this species in the wild is largely unknown and population declines have been documented (Zhao, 1998; Ministry of Science, Technology and Environment, 2000; Chan *et al.*, 2006; IUCN, 2014).

Endolymphatic sacs are terminal, intrameningeal dilations of the otic vesicle that are present in amphibians and some reptiles (Northcutt, 1979). In gekkonid species, bilateral endolymphatic ducts extend from the sacculus of the inner ear to the terminal sacs between the meninges of the brain (Bauer and Webb, 1989; Daza *et al.*, 2008). In the tokay gecko, as well as some other species, these sacs then pass through the endolymphatic foramina and expand extracranially along the cervical musculature (Bauer and Webb, 1989). Although its true function is unknown, the endolymphatic sacs are believed to serve as a storage structure for calcium and to have a role in calcium homeostasis, especially in reproductively active females (Bauer and Webb, 1989; Ineich and Gardner, 1989; Allen and Oftedal, 1993; Daza *et al.*, 2008). To our knowledge, this is the first report of neoplasia associated directly with the endolymphatic sac in a reptile.

## Case Report

An 11-year-old female tokay gecko, weighing 109 g, was presented for progressive, chronic, asymmetric swelling of the left cervical region. Mild asymmetry in the cervical region had been noted for >1 yr, but a marked progression in the swelling was observed over the 4 weeks preceding presentation. The gecko had been housed at the current facility for 10 yr with no previous pertinent medical history. Historic clutches of eggs from this female were not viable, and the most recent clutch was laid 22 months before presentation. It was housed with an adult male tokay gecko in a 90 cm × 60 cm × 90 cm fiberglass enclosure with a Plexiglas front, metal mesh screen lid, and hardwood mulch substrate. Food items were offered twice weekly and consisted of a rotation of diet items that included adult crickets dusted with a calcium powder, roaches, pinkie mice, hard-boiled eggs, and earthworms. Enclosure lighting was provided by commercially available compact fluorescent bulbs; ultraviolet spectrum lighting was not provided. Humidity was maintained at 40–60% and a temperature gradient was provided (23–33°C [70–90°F]). On physical examination, the animal was in good body condition, euhydrated, and bright, with bilateral asymmetric swellings of the cervical region. The left cervical swelling measured 3 cm in diameter and was firm on palpation, with friable overlying skin. The right cervical swelling measured 1 cm in diameter and was soft to firm on palpation. Venipuncture of the ventral coccygeal vein was performed to collect blood for hematologic and plasma biochemical analyses; results were compared to published

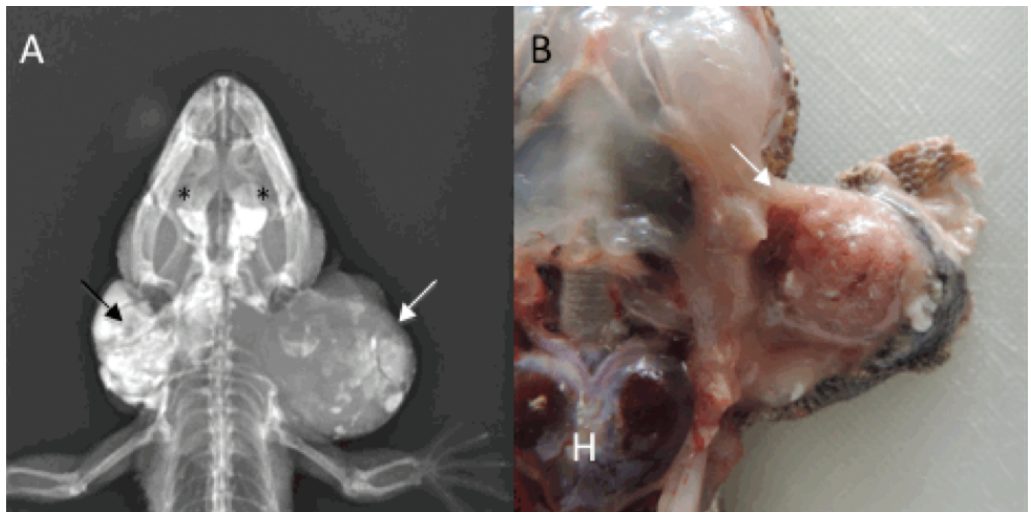


**Figure 1.** Dorsoventral radiographic image (A) and postmortem photograph (B) of the left endolymphatic sac (white arrows). (A) An eccentric, partially mineralized, soft tissue opacity mass effect is present in the left cervical region (white arrow). A diffuse mineral opacity is also present in the right endolymphatic sac (black arrow) and left and right inner ears (*). (B) A firm, pedunculated, tan mass expanded the lumen of the sac (white arrow). For reference, the heart (H) is positioned at the bottom left corner of this photograph. Bar = 1 cm.



reference values (International Species Identification System, 2002). Abnormalities included anemia (packed cell volume, 17%; reference value, 30 ± 2%), marked hypercalcemia (12.2 mM; reference value, 4.4 ± 0.1 mM), hyperphosphatemia (2.9 mM; reference value, 1.8 ± 0.7 mM), and hypoalbuminemia (1.2 μM; reference value, 3.9 μM). Whole-body right lateral and dorsoventral radiography was performed. The images revealed a markedly distended area along the left cervical region that contained an eccentric, partially mineralized, soft tissue opacity. A diffuse mineral opacity was also present along the right cervical region that extended into the right and left inner ears (Fig. 1A). The mineral opacities along both cervical regions and extending into the inner ears are consistent with the anatomic locations of the endolymphatic sacs in gekkonid species. Radiographs also revealed hyperostosis of both femurs. Fine needle aspiration of the left endolymphatic sac yielded 1 ml of white-to-pink opaque fluid. After aspiration, a firm ovoid mass was palpable, and a second fine needle aspirate was collected from the mass. An aspirate of the right endolymphatic sac revealed 0.5 ml of white opaque fluid. Aerobic culture of the fluid from both sides yielded no bacterial growth.

Direct smears from aspirates of the opaque fluid and mass within the left endolymphatic sac were stained with Wright's stain and examined. The smears were similar and contained many rectangular-to-ovoid, colorless-to-light pink crystals with pointed ends; low numbers of macrophages, containing many phagocytized crystals; and a few erythrocytes. The crystals resembled variants of calcium seen in equine urine. There were also circular-to-trabecular clusters of 20–30-μm (medium- to large-sized) round-to-polygonal cells (Fig. 2). The cells were more numerous in smears from the aspirate of the mass versus the fluid. The cells had paracentral-to-central, round-to-oval nuclei with lacey chromatin and single prominent nucleoli. They had a moderate amount of light purple cytoplasm with distinct boundaries. In some cells, the cytoplasm was lightly vacuolated to rarefied. The cells displayed mild-to-moderate anisokaryosis and anisocytosis; low numbers of binucleated cells were also identified. The cytologic results were consistent with hyperplasia or well-differentiated neoplasia of epithelial cells of the endolymphatic sac with concurrent histiocytic or granulomatous inflammation.

Given the extent of the cervical swelling and overlying skin friability, anatomic penetration of the endolymphatic sac into the meninges preventing complete surgical excision of the affected sac, and clinical pathologic abnormalities, euthanasia was elected. Euthanasia was performed by intravenous administration of pentobarbital and potassium chloride in the ventral tail vein.

On gross examination, the left endolymphatic sac was severely enlarged and contained approximately 3 ml of pink, opaque fluid and a 1.4 cm × 1.2 cm × 0.7 cm, pedunculated, firm, tan mass that projected from the lateral wall of the sac (Fig. 1B). A second, smaller (0.6 cm × 0.3 cm × 0.3 cm), sessile, firm, tan mass was present on the medial wall of the sac rostral to the pedunculated mass. The right endolymphatic sac was moderately enlarged and contained approximately 0.5 ml of opaque, white fluid. The only other significant gross finding was a 0.3-cm-diameter, thin-walled, fluid-filled cyst that projected from the capsule of the right kidney. A complete set of tissues was collected, fixed, and stored in 10% neutral buffered formalin and processed routinely for histologic examination. Paraffin sections were cut at 5 μm and stained with hematoxylin and eosin. The crystals were collected from the endolymphatic sacs and submitted for analysis (Urolithiasis Laboratory, Houston, TX; http://urolithiasis-lab.com/). Infrared X-ray crystallography, polarizing light microscopy, and acid dissolution indicated the crystals were composed of calcium carbonate.

Both masses from the left endolymphatic sac were histologically similar and characterized by papillary projections of neoplastic epithelial cells (Fig. 3A). Neoplastic cells were typically arranged in a well-organized single layer of columnar cells overlying a fine fibrovascular stroma. However, within both masses there were also multifocal areas of

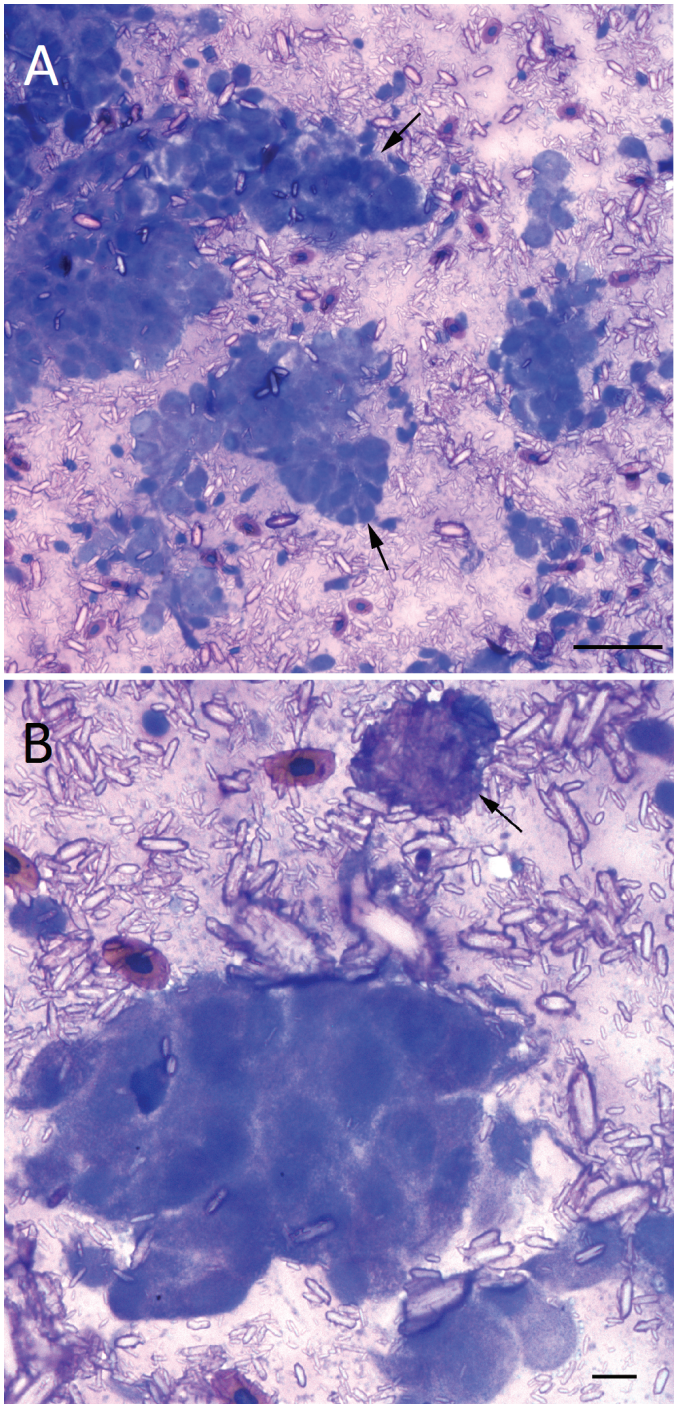


**Figure 2.** Representative photomicrographs of cytologic smears of the aspirate from the mass in the left endolymphatic sac. (A) The direct smears consisted of numerous elongate oval-to-rectangular crystals with pointed ends, a few erythrocytes, and several clusters of round to polygonal epithelial cells (arrows). The crystals were identified as calcium carbonate on further testing. Wright's stain; bar = 50 μm. (B) The epithelial cells demonstrated mild-to-moderate anisokaryosis and anisocytosis and low numbers were binucleated. A macrophage containing many phagocytized crystals is also visible in this image (arrow). Wright's stain; bar = 10 μm.

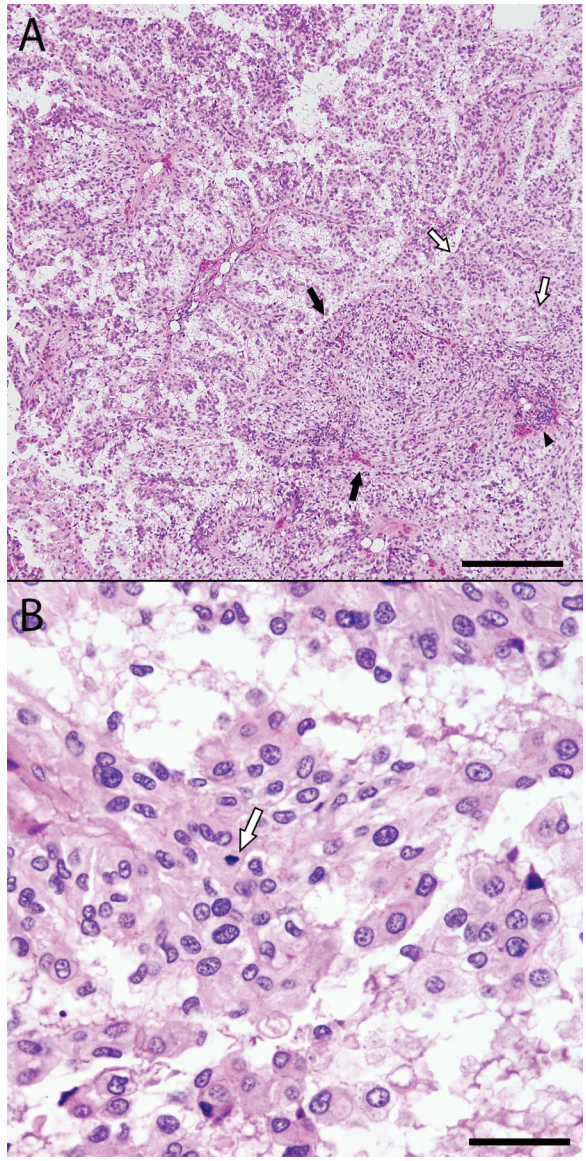


**Figure 3.** Representative photomicrographs of the endolymphatic carcinoma *in situ*. (A) Sections consisted of papillary projections of neoplastic epithelial cells that were mostly arranged as a single layer of columnar epithelial cells overlying a fine fibrovascular stroma. Multifocally within the neoplasms there was disorganization with haphazard nuclear piling (white arrows) and formations of dense islands and lobules of columnar-to-polygonal neoplastic cells (black arrows). Small numbers of mononuclear cells and granulocytes multifocally infiltrated the neoplastic stroma (black arrowhead). Hematoxylin and eosin (H&E); bar = 200 μm. (B) Neoplastic cells exhibited moderate anisocytosis and anisokaryosis with zero to four mitotic figures (white arrow) per high-magnification field. H&E; bar = 30 μm.

epithelial disorganization with haphazard piling of columnar-to-polygonal neoplastic epithelial cells that occasionally formed dense islands and lobules (Fig. 3A, arrows). Individual neoplastic epithelial cells had distinct cell borders with moderate amounts of faintly granular, variably vacuolated eosinophilic cytoplasm, and round-to-ovoid nuclei with coarsely stippled chromatin and a single, central-to-paracentral nucleolus (Fig. 3B). The neoplastic cells exhibited moderate anisocytosis and anisokaryosis, with zero to four

mitotic figures counted per × 400 magnification field. Low numbers of lymphocytes, granulocytes, and pigment-laden macrophages infiltrated the neoplastic stroma. No vascular invasion or distant metastases were noted. The degree of cellular pleomorphism and the multifocal loss of organization within the mass were consistent with a diagnosis of endolymphatic sac carcinoma *in situ*, a low grade malignant neoplasm.

The epithelium of the left endolymphatic sac surrounding the masses was multifocally hyperplastic, with the occasional formation of short, papillary projections, and variably eroded and ulcerated. Numerous macrophages distended with phagocytized basophilic-to-acicular crystals were present deep to foci of epithelial disruption, with smaller numbers of lymphocytes, plasma cells, and granulocytes that extended into the stroma of the sac. Small numbers of similar inflammatory cells and extravasated erythrocytes also extended into the cavitary lumina of the sac. The changes were consistent with chronic inflammation and epithelial hyperplasia. Fite-Faraco staining did not identify the presence of bacteria within either sac. Foci of sterile, chronic, and granulomatous and granulocytic inflammation were also present within sections of the right endolymphatic sac; hyperplastic changes, however, were rare. Other histologic findings included moderate interstitial fibrosis in both kidneys with rare urate tophi, diffuse moderate hepatic atrophy, and chronic bony remodeling of the ventral aspect of the cranial cervical vertebral bodies.

## Discussion

Scattered reports of reptile neoplasia are present throughout the literature, many of which have been reviewed by others (Hubbard *et al.*, 1983; Hernandez-Divers and Garner, 2003; Garner *et al.*, 2004; Mauldin and Done, 2006; Sykes and Trupkiewicz, 2006). In one study, 8.5% of lizards and 7.7% of geckos presenting to a specialty diagnostic service were documented with neoplasia (Garner *et al.*, 2004). In general, tumors of the hematopoietic system, skin, liver, and musculoskeleton are the most frequently reported neoplasms in lizards (Hubbard *et al.*, 1983; Hernandez-Divers and Garner, 2003; Mauldin and Done, 2006; Sykes and Trupkiewicz, 2006). Specifically in geckos, one study documented the highest prevalence of neoplastic cases as soft tissue sarcomas (1.6%, five cases) and rhabdomyosarcomas (1.3%, four cases) (Garner *et al.*, 2004). Tumors of the endolymphatic sacs are rare in humans and, to our knowledge, have not been reported in reptilian species to date (Ferri *et al.*, 2014). Carcinoma, specifically, is a malignant tumor of epithelial origin that has been associated with pathology in several reptilian species and body systems (Garner *et al.*, 2004; Mauldin and Done, 2006; Ehrhart and Powers, 2007). Given the limited understanding of neoplasia in reptiles, oncologic staging is difficult and poorly characterized (Hernandez-Divers and Garner, 2003; Mauldin and Done, 2006). In general, however, these tumors are thought to behave similar to mammalian counterparts (Hernandez-Divers and Garner, 2003; Mauldin and Done, 2006). In this case, the left endolymphatic sac contained two solid masses. Although the architecture of both masses was primarily composed of organized papilliferous projections, the presence of disorganized neoplastic foci combined with moderate cellular atypia in the absence of local or vascular invasion or distant metastases is consistent with a diagnosis of carcinoma *in situ*.

As with tumors in other species, causative factors for tumor formation in lizards is unknown. Some tumors have been associated with viruses, such as C-type virus particles within a chondrosarcoma of a corn snake (*Pantherophis guttata*), fibropapillomatosis associated with herpesvirus in green sea turtles [*Chelonia mydas*], and papovavirus (European green lizards [*Lacerta viridis*] in Bolivian side-necked turtles [*Platemys platycephala*]) (Hernandez-Divers and Garner, 2003; Mauldin and Done, 2006). There was no known etiology identified in this case; however, notably this individual had asymmetric swelling present along the cervical region for >1 yr before presentation. Diagnostically, there was evidence of mild chronic inflammation in the endolymphatic sacs. Chronic inflammation is a well-recognized predisposing factor to certain types of neoplasia in mammalian species (Henry, 2007). As such, although the observed inflammation could have been in response to the tumor itself, it is also possible that chronic inflammation over the cervical region contributed to the hyperplastic and neoplastic transformation to the gland documented in this case.

The cause of hematologic derangements in this case remains primarily speculative. Major causes of anemia include hemorrhage, hemolysis, or decreased red blood cell production (Campbell, 2006). There was no apparent significant blood loss or hemolysis. As such, the noted anemia was attributed to decreased red blood cell production, probably as a consequence of inflammatory (chronic) disease. A specific cause for the hypoalbuminemia was not identified; however, a negative acute-phase response is possible. Concurrent sources of protein loss (renal and gastrointestinal) cannot be ruled out. Protein electrophoresis would have allowed for a more accurate assessment of the plasma albumin concentration, but inadequate sample volume prohibited this testing (Campbell, 2006).

The cause of the marked hypercalcemia with milder hyperphosphatemia in this case also remains unclear. Plasma calcium and phosphorus concentrations in excess of 5 mM, as in this case, are considered increased in most reptile species, and they are most commonly due to excessive dietary supplementation of calcium, phosphorus, and vitamin $D_3$ (Campbell, 2006). Although dietary causes cannot be ruled out, this female was offered a nutritionally balanced diet supplemented with a calcium powder that did not contain vitamin $D_3$ or phosphorus and similar plasma chemistry changes have not been noted in other animals in this collection receiving a similar diet. Other differential diagnoses include much more rare conditions, including primary hyperparathyroidism, pseudohyperparathyroidism, or osteolytic bone disease, none of which were evident on postmortem evaluation (Campbell, 2006). Although reproductively active female reptiles have been documented to have a two- to four-fold increase in plasma calcium levels during follicular development, the hypercalcemia in this case is generally beyond that which would be expected in association with reproduction alone (Campbell, 2006). Furthermore, this female had not laid eggs for nearly 2 yr before presentation and ovarian follicular activity at necropsy was minimal, suggesting that reproductive status was unlikely to be the cause of the hypercalcemia in this case. Although the true function of the endolymphatic sacs remains speculative, these glands are believed to play a role

in calcium homeostasis and to serve as a calcium reservoir (Bauer and Webb, 1989; Ineich and Gardner, 1989; Allen and Oftedal, 1993; Daza *et al.*, 2008). In support of this role, reproductively active females tend to have hypertrophy of the endolymphatic sacs compared to juvenile or adult male counterparts. This trend has been documented in multiple gekkonid species, including mourning geckos (*Lepidodactylus lugubris*) and several species within the genus *Eurydactylodes* (Bauer, 1989; Ineich and Gardner, 1989). We speculate that, as a result of chronic inflammation and neoplastic transformation, the epithelial barrier of the endolymphatic sac may have been compromised, allowing for uncontrolled systemic calcium absorption. Alternatively, the neoplasm in this case could have increased systemic calcium absorption through dysregulation of normal glandular activity or even paraneoplastic production of a calcium-regulatory hormone. Furthermore, phagocytosis of the calcium crystals by macrophages noted on cytologic and histologic examination may have resulted in increased calcium resorption and contributed to the observed systemic hypercalcemia.

Reports detailing treatment options and protocols are limited in cases of reptilian neoplasia; however, radiation and intralesional chemotherapy have been attempted in some cases (Graham *et al.*, 2004; Mauldin and Done, 2006). Although the neoplasms were well demarcated in this gecko, the complex anatomy of the gekkonid endolymphatic sac and its close association with the meninges would have made complete surgical excision impossible. In addition, the small size of this patient, coupled with the advanced state of disease, would have made radiation or other local therapy options more challenging. Given the isolated masses noted within the left endolymphatic sac, it is possible that an earlier diagnosis, when these lesions were smaller, could have lent itself to surgical or other treatment options. As such, thorough evaluation of any swelling or mass on a reptile to facilitate such a diagnosis is warranted, although a balance of the risks and benefits of such an investigation must be determined on a case-by-case basis.

**Acknowledgements:** We thank the veterinarians, veterinary technicians, animal care staff, and clinical pathology technicians who provided the care and support necessary to complete this case. In particular, we thank Matt Evans for his dedication to and knowledge of care and husbandry of the tokay gecko. We also thank the Urolithiasis Laboratory for performing extensive testing to document the composition of the crystals within the endolymphatic sac.

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